REMARKS

The amendments to claim 1 involving steps (d) and (e) are supported in the specification on page 57, line 16 to page 59, line 14 and Figs. 15 and 16.

Withdrawn claims 9 to 38 were canceled. Applicant reserves his right to file a Divisional application directed to said claims.

Claims 1 to 4, 7 to 8, 42 and 43 were rejected under 35 USC 112, second paragraph, for the reasons set forth in Item No. 3 at the bottom of page 3 of the Office Action.

Step (a) of claim 1 was amended to avoid the 35 USC 112, second paragraph rejection.

It is respectfully submitted that the present claims comply with all the requirements of 35 USC 112.

The presently claimed invention concerns a method of analyzing a target nucleic acid by applying a nucleic acid amplification reaction to a test solution containing the target nucleic acid, wherein an amplified product is labeled with a marker molecule, the method comprising:

(a) performing a nucleic acid amplification reaction of the target nucleic acid in a test solution containing a forward primer and a reverse primer, a substrate comprising nucleotides, a nucleic acid polymerase and a target nucleic acid, wherein the number of one of the forward primer and the reverse primer is lower than that of the other one of the forward primer and the reverse primer, and the primer present in a lower number is labeled with a marker molecule capable of generating a detectable signal to form a labeled primer, the nucleic acid amplification reaction being performed until the primer present in a lower number is consumed;

(b) measuring a signal from the marker molecule in the test solution after initiation of the nucleic acid amplification reaction;

(c) evaluating a fluctuation motion of the labeled primer and the amplified nucleic acid which is labeled with the marker molecule, in the test solution on the basis of the signal detected;

(d) determining a number of cycles of the nucleic acid amplification reaction performed until the labeled primer has

been completely consumed, and a yield of the amplified nucleic acid which is labeled with the marker molecule based on an evaluation result of the step (c); and

(e) quantifying an initial amount of the target nucleic acid on the basis of the number of cycles of the nucleic acid amplification reaction and the yield of the amplified nucleic acid which is labeled with the marker molecule.

Claims 1 to 5, 7, 8, 39 and 42 to 43 were rejected under 35 USC 103 a being unpatentable over Salituro et al. USP 6,391,544 in view of Eigen et al. USP 5,807,677 and in view of McCabe, PCR Protocol: A Guide to Methods and Applications, (1990), pp. 76-83 for the reasons set forth in Item No. 5 on pages 3 to 5 of the Office Action.

It was admitted in the Office Action that Salituro et al. do not explicitly disclose the nucleic acid amplification reaction being performed until the primer present in a lower amount is consumed.

It was also admitted in the Office Action that Salituro et al. do not disclose evaluating a fluctuation motion of the amplified nucleic acid to quantify the target nucleic acid and

the specific ratios of the concentration of the primers as claimed in applicant's claims 42 and 43.

The presently claimed invention is directed to a method of analyzing a target nucleic acid (a template nucleic acid) contained in a test solution by means of a combination of an asymmetrical polymerase chain reaction (PCR) and a measurement of molecular fluctuation (that is, typically by fluorescence correlation spectroscopy (FCS)). In order to analyze a target nucleic acid contained in a test solution, it is important to carry out the steps (d) and (e) in the present claim 1.

More specifically, in the present invention, for example, while performing a PCR amplification reaction, two types of molecules labeled with a marker molecule (such as a fluorescent molecule), that is, the labeled primer and the amplified nucleic acid, are monitored in terms of their fluctuating movement. Since these two molecules have fluctuation characteristics that are different from each other, it is possible to determine the point when the labeled primer is consumed based on the disappearance of the signal from the labeled primer. Further, the number of cycles of the PCR executed up to that point can be

known from the record of the PCR apparatus (the thermal cycler). Furthermore, in the example where FCS is employed, the yield of the amplified nucleic acid at that point can be obtained by carrying out the calculation of the equation set forth at the middle of page 38 of the specification page 38 for "y". From these values the initial amount of the target nucleic acid (template) can be obtained based on the correlation presented in Figs. 15 and 16 of the present application.

In contrast to the presently claimed invention, none of the three references applied in the prior art rejection disclose a method of analyzing a template nucleic acid. Also, none of such three references suggest how asymmetric PCR and FCR should be combined to analyze it. Therefore, it is respectfully submitted that if these three references are combined together, the steps (d) and (e) of the present claim 1 could not be achieved or suggested.

The newly cited McCabe reference discloses a method of producing a single strand DNA from a double strand DNA by asymmetric PCR (see page 77, lines 23 to 24 of McCabe), but makes

no mention of a method for determining the initial amount of a template DNA.

It is therefore respectfully submitted that applicant's claimed invention is not rendered obvious over the references, either singly or combined in the manner relied upon in the Office Action, in view of the distinctions discussed hereinabove. It is further submitted that there are no teachings in the references to combine them in the manner relied upon in the Office Action.

Reconsideration is requested. Allowance is solicited.

If the Examiner has any comments, questions, objections or recommendations, the Examiner is invited to telephone the undersigned at the telephone number given below for prompt action.

Respectfully submitted,

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